(iv) optionally a sequence encoding a negative selection marker which is outside the homologous DNA sequences (iii).

REMARKS

In the Office Action dated April 22, 2002, claims20-40 in the above-identified U.S. patent application were rejected. Reconsideration of the rejections is respectfully requested in view of the above amendments and the following remarks.

The specification was objected to due to the format of pages 41-51. These pages have been deleted and substitute pages provided which delete the numbering of the sentences as suggested by the Examiner.

Claims 20-25, 28-31 and 35-38 were rejected under 35 USC §112, second paragraph. The claims have been amended to clarify the language found indefinite. Regarding paragraph 12 in the Office Action, applicants believe that this refers to claim 28 since claim 27 in the applicant's file does not include section (d). In view of the above claim amendments, applicants request that this rejection be withdrawn.

Claims 25-27, 32-35 and 39 were rejected under 35 USC §102(e) as anticipated by U.S. Patent No. 5,695,977. As discussed in applicant's prior response, the '977 patent is directed to a method for improving the efficiency of homologous recombination through the use of a specific recombination consensus sequence. The expression cassette disclosed in '977 contains a transcribed sequence of interest (i.e. an exogenous gene). '977 does not suggest the activation of endogenous genes as in the present invention. The claims have been amended to clarify the present invention.

In addition, the present invention is directed to a "one step" process while '977 is directed to a "two step" process. As discussed in prior responses, '977 introduces recombinase recognition sequences in the form of tandem multicopy sequences and later introduces the functional sequences in an independent process step. This two step process is difficult since recombinant clones which do not contain functional active sequences must be selected prior to the insertion of the functional sequence and thus markers must be used for selection. In

contrast to '977, in the present invention the construct contains both site specific recombinase recognition sites, which are not located next to each other (i.e. they are not tandem multicopy sequences) and a functional sequence enabling a functional selection for the desired clones. Attached to this response is a schematic drawing of the constructs according to the present invention. The general disclosure in '977 clearly does not show a construct which contains two site specific recombinase target sites which are separated by a marker gene and an expression control sequence or amplification gene (A) or activator protein binding sequences which are introduced by homologous recombination (B) or a test system for non-coding 5' and/or 3' sequences using a heterologous expression control sequence operatively linked to a reporter gene (C). The office action contends that the two step process disclosed in '977 is only one embodiment of '977 and is not the only possible method. Applicants were unable to find any disclosure in '977 which suggests a method other than the above discussed two step process. If this rejection is to be maintained, applicants request that the disclosure relied on by the Examiner be specifically pointed out. In view of the above discussion, applicants contend that the present claims are not anticipated by '977 and request that this rejection be withdrawn.

Claims 36-40 were rejected under 35 USC §103(a) as unpatentable over U.S. Patent No. 6,020,144 in view of Cruz. U.S. Patent No. 6,020,144 discloses a process for obtaining DHFR negative trypansomal cells and Cruz teaches the replacement of DHFR in a protozoan parasite. The present claims recite mammalian cells and vectors for expression in mammalian cells. In view of the fact that neither U.S. Patent No. 6,020,144 or Cruz disclose mammalian cells or vectors for expression in mammalian cells, applicants contend that claims 36-40 are patentable over the cited prior art and request that this rejection be withdrawn.

Claims 20-28 and 30-35 were rejected under 35 USC §103(a) as unpatentable over U.S. Patent No. 5,695,977 in view of WO 94/12650 and U.S. Patent No. 6,130,364. As discussed above, '977 does not suggest the activation of endogenous genes or a one step process as in the present invention. U.S. Patent No. 6,130,364 does not cure this deficiency as '364 does not disclose or suggest a one step process either. Column 12, lines 42-48, pointed out in the office action discloses a homology targeting vector which contains a single targeting sequence, a lox site and a selectable marker gene. This vector is also discussed at column 5, lines 37-44. Column 14, lines 8-18, indicates that the lox sites are first introduced and then the

modifying sequence is introduced. Thus, '364 clearly indicates that a second lox targeting vector (as discussed at column 12, lines 52-55) is required for the recombination. WO 94/12650 does not cure the deficiencies in Patent No. 5,695,977 and U.S. Patent No. 6,130,364 as WO 94/12650 does not suggest or disclose a one step site specific recombination process either. Page 12, line 26 to page 13, line 33, teaches that two separate DNA constructs are used. One construct is introduced upstream and one is introduced downstream. In view of the fact that none of the cited references individually or in combination suggest or disclose a one step site specific recombination process, applicants request that this rejection be withdrawn.

Claims 20-35 were rejected under 35 USC §103(a) as unpatentable over U.S. Patent No. 5,695,977 in view of WO 94/12650 and U.S. Patent No. 6,130,364 further in view of WO 97/37012. As discussed above, U.S. Patent No. 5,695,977, WO 94/12650 and U.S. Patent No. 6,130,364 do not suggest or disclose a one step site specific recombination process. WO 97/37012 does not cure this deficiency as WO 97/37012 does not suggest or disclose a one step site specific recombination process or site specific recombinase recognition sites. In view of these deficiencies, applicants request that this rejection be withdrawn.

Applicants respectfully submit that all of claims 20-43 are now in condition for allowance. If it is believed that the application is not in condition for allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below so that an interview can be scheduled.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper, may be charged to Counsel's Deposit Account No. 02-2135.

RESPECTFULLY SUBMITTED,					
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Enclosures: Replacement pages 41-51
Marked up pages 41-51
Marked up claims
Schematic Drawing

APPENDIX 1

Marked-Up copy of claims to show amendments:

20. (Twice Amended) A process for changing the expression of a nucleic acid sequence which is present endogenously in a eukaryotic cell, the method comprising

(a) transfecting the cell with a vector comprising the following sequences

(i) at least one sequence, which upon expression is capable of changing the expression of the nucleic acid sequence which is present endogenously in the cell, and selected from the group consisting of a heterologous expression control sequence and an amplification gene,

(ii)a sequence encoding a positive selection marker,

(iii) at least two target sequences for a site-specific recombinase flanking the sequences of (i) and (ii), and

(iv) DNA sequences which flank the sequences of (i), (ii) and (iii) and are homologous to a nucleic acid section in the genome of the cell in order to allow a homologous recombination,

(b) culturing the transfected cell under conditions under which a homologous recombination of the vector takes place,

(c) isolating the cell obtained according to step (b) wherein the expression of the nucleic acid sequence which is present endogenously is under control of the at least one sequence (i), and

(d) expressing the [at least one sequence of (i) to thereby change the expression of the nucleic acid sequence which is present endogenously in the cell] nucleic acid sequence which is present endogenously in the cell under control of the at least one sequence of (i) to thereby determine changes in the expression.

24. (Twice Amended) A vector suitable for homologous recombination, comprising the following sequences

(i) at least one sequence selected from the group consisting of an expression control sequence and an amplification gene each of which [upon expression] is capable of changing the expression of the nucleic acid sequence which is present endogenously in the cell,

(ii) a sequence encoding a positive selection marker,

(iii) at least two target sequences for a site-specific recombinase flanking the sequences of (i) and (ii), and

(iv) DNA sequences which flank the sequences of (i), (ii) and (iii) and are homologous to a nucleic acid section in the genome of a cell in order to allow a homologous recombination, and

(v) optionally a sequence encoding a negative selection marker.

25. (Twice Amended) A vector, comprising

(i) at least one sequence selected from the group consisting of a heterologous expression control sequence and an amplification gene each of which [upon expression] is capable of changing the expression of the nucleic acid sequence which is present endogenously in the cell,

(ii) a sequence encoding a positive selection marker,

(iii) at least two recombinase target sequences flanking the sequences of (i) and (ii), and

(iv) optionally a sequence encoding a negative selection marker.

28. (Twice Amended) A process for changing the expression of a nucleic acid sequence which is present endogenously in a eukaryotic cell, the method comprising

(a) transfecting the cell with a vector comprising

(i) at least one nucleic acid sequence which binds an activator protein,

(ii) a sequence encoding a positive selection marker, and

(iii) DNA sequences which flank the sequences of (i) and (ii) and are

homologous to a nucleic acid section in the genome of the cell in order to allow a homologous recombination,

(b) culturing the transfected cell under conditions under which a homologous recombination of the vector takes place,

(c) isolating the cell obtained according to step (b), and

(d) expressing the [sequence of (i)] nucleic acid sequence which is present endogenously in the cell under the control of the at least one sequence of (i) under conditions under which the activator protein is bound thereby changing the expression of the nucleic acid sequence which is present endogenously in the cell.

35. (Twice Amended) A process for testing the influence of non-coding nucleic acid sequences from the region of a target gene present endogenously in a eukaryotic cell on its expression, the process comprising

(a) transfecting the cell with a vector comprising

(i) a heterologous expression control sequence which is active or can be activated in the cell and is operatively linked with a reporter gene, and

(ii) non-coding nucleic acid sequences on the 5'-side and/or the 3'-side from the region of the target gene which are capable of influencing the expression of the target gene and the reporter gene,

(b) culturing the cell under conditions under which the heterologous expression control sequence is active, and

(c) measuring expression of the reporter gene as an indication of the influence of said non-coding nucleic acid sequences on the target gene.

36. (Twice Amended) A process for obtaining a DHFR-negative [eukaryotic] mammalian cell, the process comprising

(a) transfecting a DHFR-positive mammalian cell with a first vector comprising

(i) at least one [DHFR-negative] target sequence for a site-specific recombinase;

(ii) DNA sequences which flank sequence (i) and are homologous to a DHFR nucleic acid sequence which is present endogenously in the cell in order to allow a homologous recombination,

(iii) optionally a sequence encoding a first positive selection marker, and

(iv) optionally a sequence encoding a negative selection marker,

(b) culturing the transfected cell under conditions under which a homologous recombination of the vector takes place thereby incorporating the DHFR-negative target sequence into the DHFR-positive mammalian cell to create a DHFR-negative cell, and

(c) isolating the cells obtained according to step (b) to obtain a DHFR-negative [eukaryotic] mammalian cell.

37. (Twice Amended) A process for obtaining a [eukaryotic] mammalian cell containing a nucleic acid sequence to be amplified and a heterologous DHFR gene, the process comprising

(a) obtaining a DHFR-negative [eukaryotic] mammalian cell by the process as claimed in claim 36,

(b) transfecting the cell of step (a) with a second vector comprising

(i) a nucleic acid sequence coding for a DHFR,

(ii) a nucleic acid sequence to be amplified which codes for a protein in an expressible form,

(iii) optionally a sequence encoding a second positive selection marker, and

(iv) at least two recombinase target sequences flanking the sequences of (i), (ii) and (iii), if present,

(c) culturing the transfected cell under conditions under which the sequences of (i), (ii) and (iii), if present, are integrated into the recombinase target sequence that is already present in the genome of the cell, and

(d) isolating the cell obtained according to step (c) to obtain a [eukaryotic] mammalian cell containing a nucleic acid sequence to be amplified and a heterologous DHFR gene.

39. (Twice Amended) A vector for expression in mammalian cells, comprising

(i) a nucleic acid sequence coding for a DHFR,

(ii) a nucleic acid sequence to be amplified which codes for a protein in an expressible form,

(iii) optionally a sequence encoding a positive selection marker, and

(iv) at least two recombinase target sequences flanking the sequences of (i), (ii) and (iii), if present.

40. (Twice Amended) A vector for expression in mammalian cells, wherein said vector is suitable for homologous recombination, comprising

(i) optionally a sequence encoding a positive selection marker,

(ii) at least one recombinase target sequence which flanks the sequence of (i), if present,

(iii) DNA sequences which flank the sequences of (i), if present, and (ii) and which are homologous to a DHFR nucleic acid sequence which is present endogenously in a cell in order to allow a homologous recombination, and

(iv) optionally a sequence encoding a negative selection marker which is outside the homologous DNA sequences (iii).

APPENDIX 2

Replacement pages 41-51

Preferred embodiments of the invention are shown in the following as part of the description:

Process for changing the expression of a nucleic acid sequence which is present endogenously in a eukaryotic cell,

wherein

(a) the cell is transfected with a first vector comprising
  (i) at least one sequence selected from a first heterologous expression control sequence and a first amplification gene,
  (ii) a positive selection marker gene,
  (iii) at least two target sequences for a site-specific recombinase flanking the sequences (i) and (ii),
  (iv) DNA sequences which flank the sequences (i), (ii) and (iii) and are homologous to a nucleic acid section in the genome of the cell in order to allow a homologous recombination

(b) the transfected cell is cultured under conditions under which a homologous recombination of the vector takes place and

(c) the cell obtained according to step (b) is isolated.

The process discussed above

wherein

loxP sequences are used as recombinase target sequences.

The process as discussed above,
wherein
the cell is a human cell.

The process as discussed above,
wherein
the cell is an immortalized cell.

The process as discussed above,
wherein
the cell is a HT1080, Namalwa or HeLa S3 cell.

The process as discussed above,
wherein
the heterologous expression control sequence contains a promoter/enhancer preferably a viral promoter and particularly preferably a CMV promoter.

The process as discussed above,
wherein
the heterologous expression control sequence contains a 3' non-coding sequence.

The process as discussed above,
wherein
the homologous sequences are selected such that an endogenous expression control sequence of the nucleic acid sequence that is present endogenously is removed by homologous recombination.

The process as discussed above,
wherein
the positive selection marker gene is a neomycin, kanamycin, geneticin of hygromycin resistance gene.

The process as discussed above,
wherein
the vector additionally contains a negative selection marker gene which is arranged outside the homologous sequences as claimed in claim 1(a) (iv).

The process as discussed above,
wherein
the nucleic acid sequence that is located between the recombinase target sequences is cut out of the genome of the cell by transient activation of a site-specific recombinase that recognizes the target sequences.

The process according to item 1,
wherein

(a) the cell is transfected with a further vector comprising
(i) at least one sequence selected from a second heterologous expression control sequence and a second amplification gene
(ii) a positive selection marker gene which preferably differs from the positive selection marker gene of the first vector and
(iii) at least two recombinase target sequences flanking the sequences (i) and (ii)
(b) the transfected cell is cultured under conditions under which the sequence flanked by the target

sequences is integrated into the target sequence in the genome of the cell

(c) the cell obtained according to step (b) is isolated and

(d) optionally steps (a) to (c) are repeated at least once with expression control sequences or/and amplification genes which vary in each case.

Vector for homologous recombination comprising,

(i) at least one sequence selected from an expression control sequence and an amplification gene,
(ii) a positive selection marker gene,
(iii) at least two target sequences for a site-specific recombinase which flank the sequences (i) and (ii),
(iv) DNA sequences flanking the sequences (i), (ii) and (iii) which are homologous to a nucleic acid section in the genome of a cell in order to allow a homologous recombination and
(v) optionally a negative selection marker gene.

Vector comprising

(i) at least one sequence selected from a heterologous expression control sequence and an amplification gene,
(ii) a positive selection marker gene,
(iii) at least two recombinase target sequences which flank the sequences (i) and (ii),
(iv) optionally a negative selection marker gene.

Eukaryotic cell, preferably a human cell obtainable by a process as discussed in one of the items 1 to 12.

Eukaryotic cell, preferably a human cell,
wherein
it

(a) contains at least one chromosomally located sequence selected from a heterologous expression control sequence and an amplification gene in operative linkage with a nucleic acid sequence that is present endogenously and

(b) this sequence is flanked by recombinase target sequences.

Process for changing the expression of a nucleic acid sequence that is present endogenously in a eukaryotic cell,
wherein

(a) the cell is transfected with a vector comprising

(i) at least one nucleic acid sequence that binds an activator protein,

(ii) a positive selection marker gene,

(iii) DNA sequences flanking the sequences (i) and (ii) which are homologous to a nucleic acid section in the genome of the cell in order to allow a homologous recombination,

(b) the transfected cell is cultured under conditions under which a homologous recombination of the vector takes place and

(c) the cell obtained according to step (b) is isolated.

The process as discussed above,
wherein
at least one hypoxia-inducible-factor-(HIF)-binding nucleic acid sequence is used.

The process as discussed above,
wherein
the HIF-binding nucleic acid sequence is selected from the 53 bp sequence according to sequence ID NO.1, from the 43 bp sequence according to sequence ID NO.2, a sequence that is homologous to these sequences or a sequence which hybridizes with these sequences under stringent conditions.

The process as discussed above additionally comprising transfecting the cell with a vector comprising

(i) a nucleic acid sequence coding for an activator protein which is operatively linked with an active expression control sequence in this cell and
(ii) optionally a positive selection marker gene.

The process as discussed above,
wherein
the activator protein is a HIF-1α or/and a HIF-1β protein.

The process as discussed above,
wherein
the cells are cultured at an O_2 concentration of 0.1 to 2 %.

Vector for homologous recombination, comprising

(i) at least one nucleic acid sequence which binds an activator protein,
(ii) a positive selection marker gene,
(iii) DNA sequences flanking the sequences (i) and (ii) which are homologous to a nucleic acid

section in the genome of the cell in order to allow a homologous recombination.

Eukaryotic cell, preferably a human cell obtainable by a process as discussed above.

Eukaryotic cell, preferably a human cell, wherein

it contains at least one heterologous, chromosomally localized, nucleic acid fragment that binds an activator protein/activator protein complex which is operatively linked with a gene that is present endogenously in the cell.

Process for testing the influence of non-coding

nucleic acid sequences from the region of a target

gene present endogenously in a eukaryotic cell on its expression which is characterized in that

(a) the cell is transfected with a vector comprising

(i) a heterologous expression control sequence that is active or can be activated in the cell which is operatively linked with a reporter gene and

(ii) non-coding nucleic acid fragments on the 5' side or/and 3' side from the region of the target gene,

(b) the cell is cultured under conditions under which the expression control sequence is active and

(c) the expression of the reporter gene is measured.

The process as discussed above,
wherein
the reporter gene codes for chloroamphenicol acetyl transferase (CAT), β-galactosidase (β-Gal) or lacZ.

The process as discussed above,
wherein

(a) at least 2 vectors which contain 5' or/and 3' non-coding nucleic acid fragments of the target gene that are different from each other, are transfected into different cells in each case and
(b) the expression of the reporter gene is determined in the different cells.

Process for providing a DHFR-negative eukaryotic cell, **wherein**

(a) the cell is transfected with a first vector comprising
(i) at least one target sequence for a site- specific recombinase,
(ii) DNA sequences flanking sequence (i) which are homologous to a DHFR nucleic acid sequence that is present endogenously in the cell in order to allow a homologous recombination and
(iii) optionally a positive selection marker gene and optionally a negative selection marker gene,
(b) the transfected cell is cultured under conditions under which a homologous recombination of the vector takes place and
(c) the cell obtained according to step (b) is isolated.

The process as discussed above,
wherein

loxP sequences are used as the recombinase target sequences.

The process as discussed above,
wherein
the nucleic acid sequence coding for the positive selection marker gene is a neomycin, kanamycin, geneticin or hygromycin resistance gene.

The process as discussed above,
wherein
the nucleic acid sequence coding for the negative selection marker gene is a thymidine kinase gene (TK) or/and hypoxanthine-guanine-phosphoribosyltransferase gene (HGPRT).

The process as discussed above,
wherein
the sequence that is flanked by the recombinase target sequences is cut out of the genome of the cell by transient activation of the corresponding recombinase.

Process for introducing a heterologous DHFR gene into a eukaryotic cell,
wherein
a cell obtained by the above process

(a) is transfected with a third vector comprising

(i) optionally a positive selection marker gene which preferably differs from the positive selection marker gene of the first vector,

(ii) a nucleic acid sequence coding for a DHFR,

(iii) a nucleic acid sequence to be amplified coding for a protein in which each nucleic acid sequences from the partial sequences (i), (ii) and (iii) is flanked on the 5' side and 3' side by at least one recombinase target sequence,

(b) the transfected cell is cultured under conditions under which the nucleic acid sequence flanked by recombinase target sequences is integrated into the recombinase target sequence that is already
present in the genome of the cell and

(c) the cell obtained according to step (b) is isolated.

Vector, comprising

(i) optionally a positive selection marker gene,

(ii) a nucleic acid sequence coding for a DHFR and

(iii) a nucleic acid sequence in an expressible form coding for a desired protein

in which each nucleic acid sequence from the partial sequences (i), (ii) and (iii) is flanked on the 5'
side and 3' side by at least one recombinase target sequence.

Vector for homologous recombination comprising,

(i) optionally a positive selection marker gene

(ii) at least one recombinase target sequence in each case which flanks the sequence (i),

(iii) DNA sequences flanking the sequences (i) and

(ii) which are homologous to a DHFR nucleic acid sequence that is present endogenously in a cell

in order to allow a homologous recombination and

(iv) optionally a negative selection marker gene outside the homologous sequences (iii).

Eukaryotic cell, preferably a human cell obtainable by a process as discussed above.

Eukaryotic cell, preferably a human cell,
wherein

(a) at least one endogenous nucleic acid sequence coding for a DHFR is inactivated and

(b) at least one recombinase target sequence is

integrated into the genome in the region of this nucleic acid sequence coding for DHFR.

Eukaryotic cell, preferably a human cell,

characterized by

a heterologous nucleic acid sequence in the region of an endogenous DHFR gene locus, comprising

(i) a nucleic acid sequence coding for DHFR,

(ii) a nucleic acid sequence coding for a desired protein and

(iii) at least one recombinase target sequence.

APPENDIX 3

Marked-Up copy of specification pages 41-51 to show amendments

Preferred embodiments of the invention are shown in the following as part of the description:

~~1.~~ Process for changing the expression of a nucleic acid sequence which is present endogenously in a eukaryotic cell,

wherein

(a) the cell is transfected with a first vector comprising
(i) at least one sequence selected from a first heterologous expression control sequence and a first amplification gene,
(ii) a positive selection marker gene,
(iii) at least two target sequences for a site-specific recombinase flanking the sequences (i) and (ii),
(iv) DNA sequences which flank the sequences (i), (ii) and (iii) and are homologous to a nucleic acid section in the genome of the cell in order to allow a homologous recombination

(b) the transfected cell is cultured under conditions under which a homologous recombination of the vector takes place and

(c) the cell obtained according to step (b) is isolated.

~~2. Process as claimed in item 1,~~ The process discussed above
wherein

loxP sequences are used as recombinase target sequences.

~~3. Process as claimed in item 1 or 2,~~ The process as discussed above, **wherein**

the cell is a human cell.

~~4. Process as claimed in one of the previous items,~~ The process as discussed above, **wherein**

the cell is an immortalized cell.

~~5. Process as claimed in item 4,~~ The process as discussed above, **wherein**

the cell is a HT1080, Namalwa or HeLa S3 cell.

~~6. Process as claimed in one of the previous items,~~ The process as discussed above, **wherein**

the heterologous expression control sequence contains a promoter/enhancer preferably a viral promoter and particularly preferably a CMV promoter.

~~7. Process as claimed in one of the items 1 to 6,~~ The process as discussed above, **wherein**

the heterologous expression control sequence contains a 3' non-coding sequence.

~~8. Process as claimed in one of the previous items,~~ The process as discussed above, **wherein**

the homologous sequences are selected such that an endogenous expression control sequence of the nucleic acid sequence that is present endogenously is removed by homologous recombination.

~~9. Process as claimed in one of the previous items,~~ The process as discussed above,
wherein
the positive selection marker gene is a neomycin, kanamycin, geneticin of hygromycin resistance gene.

~~10. Process as claimed in one of the previous items,~~ The process as discussed above,
wherein
the vector additionally contains a negative selection marker gene which is arranged outside the homologous sequences as claimed in claim 1(a) (iv).

~~11. Process as claimed in one of the previous items,~~ The process as discussed above,
wherein
the nucleic acid sequence that is located between the recombinase target sequences is cut out of the genome of the cell by transient activation of a site-specific recombinase that recognizes the target sequences.

~~12. Process as claimed in item 1,~~ The process according to item 1,
wherein

(a) the cell is transfected with a further vector comprising
(i) at least one sequence selected from a second heterologous expression control sequence and a second amplification gene
(ii) a positive selection marker gene which preferably differs from the positive selection marker gene of the first vector and
(iii) at least two recombinase target sequences flanking the sequences (i) and (ii)

(b) the transfected cell is cultured under conditions under which the sequence flanked by the target

sequences is integrated into the target sequence in the genome of the cell

(c) the cell obtained according to step (b) is isolated and

(d) optionally steps (a) to (c) are repeated at least once with expression control sequences or/and amplification genes which vary in each case.

~~13.~~ Vector for homologous recombination comprising,

(i) at least one sequence selected from an expression control sequence and an amplification gene,
(ii) a positive selection marker gene,
(iii) at least two target sequences for a site-specific recombinase which flank the sequences (i) and (ii),
(iv) DNA sequences flanking the sequences (i), (ii) and (iii) which are homologous to a nucleic acid section in the genome of a cell in order to allow a homologous recombination and
(v) optionally a negative selection marker gene.

~~14.~~ Vector comprising
(i) at least one sequence selected from a heterologous expression control sequence and an amplification gene,
(ii) a positive selection marker gene,
(iii) at least two recombinase target sequences which flank the sequences (i) and (ii),
(iv) optionally a negative selection marker gene.

~~15.~~ Eukaryotic cell, preferably a human cell obtainable by a process as ~~claimed~~ discussed in one of the items 1 to 12.

~~16.~~ Eukaryotic cell, preferably a human cell,
wherein
it

(a) contains at least one chromosomally located sequence selected from a heterologous expression control sequence and an amplification gene in operative linkage with a nucleic acid sequence that is present endogenously and

(b) this sequence is flanked by recombinase target sequences.

~~17.~~ Process for changing the expression of a nucleic acid sequence that is present endogenously in a eukaryotic cell,
wherein

(a) the cell is transfected with a vector comprising

(i) at least one nucleic acid sequence that binds an activator protein,

(ii) a positive selection marker gene,

(iii) DNA sequences flanking the sequences (i) and (ii) which are homologous to a nucleic acid section in the genome of the cell in order to allow a homologous recombination,

(b) the transfected cell is cultured under conditions under which a homologous recombination of the vector takes place and

(c) the cell obtained according to step (b) is isolated.

~~18. Process as claimed in item 17,~~ The process as discussed above,
wherein
at least one hypoxia-inducible-factor-(HIF)-binding nucleic acid sequence is used.

~~19. Process as claimed in item 18,~~ The process as discussed above, **wherein**
the HIF-binding nucleic acid sequence is selected from the 53 bp sequence according to sequence ID NO.1, from the 43 bp sequence according to sequence ID NO.2, a sequence that is homologous to these sequences or a sequence which hybridizes with these sequences under stringent conditions.

~~20. Process as claimed in one of the items 17 to 19~~ The process as discussed above additionally comprising transfecting the cell with a vector comprising

(i) a nucleic acid sequence coding for an activator protein which is operatively linked with an active expression control sequence in this cell and
(ii) optionally a positive selection marker gene.

~~21. Process as claimed in item 20,~~ The process as discussed above, **wherein**
the activator protein is a HIF-1α or/and a HIF-1β protein.

~~22. Process as claimed in one of the items 18 to 21,~~ The process as discussed above, **wherein**
the cells are cultured at an O_2 concentration of 0.1 to 2 %.

~~23.~~ Vector for homologous recombination, comprising

(i) at least one nucleic acid sequence which binds an activator protein,
(ii) a positive selection marker gene,
(iii) DNA sequences flanking the sequences (i) and (ii) which are homologous to a nucleic acid

section in the genome of the cell in order to allow a homologous recombination.

~~24.~~ Eukaryotic cell, preferably a human cell obtainable by a process as ~~claimed in one of the items 17 to 21.~~ discussed above.

~~25.~~ Eukaryotic cell, preferably a human cell, wherein

it contains at least one heterologous, chromosomally localized, nucleic acid fragment that binds an activator protein/activator protein complex which is operatively linked with a gene that is present endogenously in the cell.

~~26.~~ Process for testing the influence of non-coding

nucleic acid sequences from the region of a target

gene present endogenously in a eukaryotic cell on its expression which is characterized in that

(a) the cell is transfected with a vector comprising

(i) a heterologous expression control sequence that is active or can be activated in the cell which is operatively linked with a reporter gene and

(ii) non-coding nucleic acid fragments on the 5' side or/and 3' side from the region of the target gene,

(b) the cell is cultured under conditions under which the expression control sequence is active and

(c) the expression of the reporter gene is measured.

~~27. Process as claimed in item 26,~~ The process as discussed above, **wherein**

the reporter gene codes for chloroamphenicol acetyl transferase (CAT), β-galactosidase (β-Gal) or lacZ.

~~28. Process as claimed in one of the items 26 or 27,~~The process as discussed above,
wherein

(a) at least 2 vectors which contain 5' or/and 3' non-coding nucleic acid fragments of the target gene that are different from each other, are transfected into different cells in each case and
(b) the expression of the reporter gene is determined in the different cells.

~~29.~~ Process for providing a DHFR-negative eukaryotic cell, **wherein**

(a) the cell is transfected with a first vector comprising
(i) at least one target sequence for a site- specific recombinase,
(ii) DNA sequences flanking sequence (i) which are homologous to a DHFR nucleic acid sequence that is present endogenously in the cell in order to allow a homologous recombination and
(iii) optionally a positive selection marker gene and optionally a negative selection marker gene,
(b) the transfected cell is cultured under conditions under which a homologous recombination of the vector takes place and
(c) the cell obtained according to step (b) is isolated.

~~30. Process as claimed in item 29,~~ The process as discussed above, **wherein**

loxP sequences are used as the recombinase target sequences.

~~31. Process as claimed in one of the items 29 or 30,~~ The process as discussed above,
wherein
the nucleic acid sequence coding for the positive selection marker gene is a neomycin, kanamycin, geneticin or hygromycin resistance gene.

~~32. Process as claimed in one of the items 29 to 31,~~ The process as discussed above,
wherein
the nucleic acid sequence coding for the negative selection marker gene is a thymidine kinase gene (TK) or/and hypoxanthine-guanine-phosphoribosyltransferase gene (HGPRT).

~~33. Process as claimed in one of the items 29 to 32,~~The process as discussed above,
wherein
the sequence that is flanked by the recombinase target sequences is cut out of the genome of the cell by transient activation of the corresponding recombinase.

~~34.~~ Process for introducing a heterologous DHFR gene into a eukaryotic cell,
wherein
a cell obtained by the above process ~~according to item 33~~
(a) is transfected with a third vector comprising
(i) optionally a positive selection marker gene which preferably differs from the positive selection marker gene of the first vector,
(ii) a nucleic acid sequence coding for a DHFR,
(iii) a nucleic acid sequence to be amplified coding for a protein in which each nucleic acid sequences from the partial sequences (i), (ii) and (iii) is flanked on the 5' side and 3' side by at least one recombinase target sequence,

(b) the transfected cell is cultured under conditions under which the nucleic acid sequence flanked by recombinase target sequences is integrated into the recombinase target sequence that is already
present in the genome of the cell and

(c) the cell obtained according to step (b) is isolated.

~~35.~~ Vector, comprising

(i) optionally a positive selection marker gene,

(ii) a nucleic acid sequence coding for a DHFR and

(iii) a nucleic acid sequence in an expressible form coding for a desired protein

in which each nucleic acid sequence from the partial sequences (i), (ii) and (iii) is flanked on the 5'
side and 3' side by at least one recombinase target sequence.

~~36.~~ Vector for homologous recombination comprising,

(i) optionally a positive selection marker gene

(ii) at least one recombinase target sequence in each case which flanks the sequence (i),

(iii) DNA sequences flanking the sequences (i) and

(ii) which are homologous to a DHFR nucleic acid sequence that is present endogenously in a cell

in order to allow a homologous recombination and

(iv) optionally a negative selection marker gene outside the homologous sequences (iii).

~~37.~~ Eukaryotic cell, preferably a human cell obtainable by a process ~~according to one of the items 29 to 34.~~ <u>as discussed above.</u>

~~38.~~ Eukaryotic cell, preferably a human cell,
wherein

(a) at least one endogenous nucleic acid sequence coding for a DHFR is inactivated and

(b) at least one recombinase target sequence is

integrated into the genome in the region of this nucleic acid sequence coding for DHFR.

~~39.~~ Eukaryotic cell, preferably a human cell,

characterized by

a heterologous nucleic acid sequence in the region of an endogenous DHFR gene locus, comprising

(i) a nucleic acid sequence coding for DHFR,

(ii) a nucleic acid sequence coding for a desired protein and

(iii) at least one recombinase target sequence.